Candida tibetensis sp. nov. and *Candida linzhiensis* sp. nov., novel anamorphic, ascomycetous yeast species from Tibet

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Three anamorphic, ascomycetous yeast strains isolated from plant samples collected in Linzhi District, Tibet, China, were revealed as representing two novel species by 26S rRNA gene D1/D2 domain sequence and physiological property comparisons. The names *Candida tibetensis* sp. nov. and *Candida linzhiensis* sp. nov. are proposed for these novel species, with XZ 41-6^T (= AS 2.3072^T = CBS 10298^T) and XZ 92-1^T (= AS 2.3073^T = CBS 10299^T) as the respective type strains. D1/D2 sequence analysis showed that *C. tibetensis* and *C. linzhiensis* are closely related to *Candida caryicola* and *Candida sequanensis*, respectively.

Candida tanzawaensis and *Candida sequanensis* were among the species of ascomycetous yeasts that were on isolated branches in the phylogenetic trees drawn from 26S rRNA gene D1/D2 domain sequences (Kurtzman & Robnett, 1997, 1998). Recently, more than 20 species with close phylogenetic relationships to *C. tanzawaensis* were described (Kurtzman, 2001; Suh *et al.*, 2004). Most of the species were isolated from sources associated with insects. Consequently, the *C. tanzawaensis* clade has been expanded substantially. However, no close relatives of *C. sequanensis* have been reported.

In an investigation on Tibetan yeast diversity, two novel anamorphic, ascomycetous yeast species were identified. One of them was closely related to *C. sequanensis*, and the other to *Candida caryicola*. *C. caryicola* was described by Kurtzman (2001) as a species weakly associated with the *C. tanzawaensis* clade.

The yeast strains examined were isolated by an enrichment method using YM medium containing penicillin (100 µg ml⁻¹) and tetracycline (25 µg ml⁻¹) to inhibit the growth of bacteria. Strains XZ 41-6^T, XZ 69C3 and XZ 92-1^T were isolated from the flowers, leaves and fruit, respectively, of unidentified plants. These samples were collected in July 2004 from Linzhi District, Tibet, at altitudes of 3100–3300 m.

Most of the morphological, physiological and biochemical characteristics were examined according to standard

methods (Yarrow, 1998). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986). Extraction, purification and identification of ubiquinones were carried out according to Yamada & Kondo (1973).

Nuclear DNA was extracted by the method of Makimura *et al.* (1994). The DNA fragment covering the internal transcribed spacer region (including 5·8S rDNA) and the 26S rDNA D1/D2 domain was amplified and sequenced by using a method described previously (Lu *et al.*, 2004). Sequences were aligned with the program CLUSTAL X (Thompson *et al.*, 1997). Molecular phylogenetic analysis was performed by using the methods described by Bai *et al.* (2002). Reference sequences were retrieved from GenBank under the accession numbers indicated in the tree shown in Fig. 1.

Strains XZ 41-6^T and XZ 69C3 have identical D1/D2 and internal transcribed spacer sequences. In the phylogenetic tree drawn from the D1/D2 sequence alignment (Fig. 1), the two strains clustered together with *C. caryicola*. They differed from *C. caryicola* by 5·2 % mismatches (25 substitutions and 4 indels) in the D1/D2 domain. Strain XZ 92-1^T was closely related to *C. sequanensis* (Fig. 1). This strain differed from *C. sequanensis* by 1·8 % (10 substitutions) nucleotide divergence in the D1/D2 domain. Another close relative of strain XZ 92-1^T shown in the tree was *Candida melibiosacea*, which was recently reinstated as a distinct species by Daniel (2003), with *Trichosporon melibiosaceum* – a former synonym of *Candida fennica* (Meyer *et al.*, 1984) – as the basionym.

The novel *Candida* species described by Kurtzman (2001) and Suh *et al.* (2004), with the exception of *C. caryicola*, formed a strongly supported clade with *C. tanzawaensis*. *C. caryicola* was described as a species basal to the *C.*

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The GenBank/EMBL/DDBJ accession numbers for the internal transcribed spacer and the 26S rRNA gene D1/D2 domain sequences of strains XZ 41-6^T and XZ 92-1^T are DQ269920 and DQ269921, respectively.

Fig. 1. Phylogenetic tree drawn from neighbour-joining analysis of 26S rRNA gene D1/D2 domain sequences, depicting the relationship of the two novel *Candida* species with respect to closely related taxa. Bootstrap percentages above 50 % (from 1000 bootstrap replicates) are shown. Reference sequences were retrieved from GenBank under the accession numbers indicated. Bar, 0.02 K_{nuc} units.

tanzawaensis clade and weakly associated with the clade (Kurtzman, 2001). However, with the addition of novel species to the existing clades, the present study shows that *C. caryicola* appears to be more closely related to the clade represented by *C. sequanensis* than to the *C. tanzawaensis* clade (Fig. 1).

In agreement with their identical D1/D2 and internal transcribed spacer sequences, strains XZ 41-6^T and XZ 69C3 were similar in terms of morphological and physiological characteristics, indicating that they are conspecific. They differed from the phylogenetically closest relative, *C. caryicola*, in the assimilation reactions with cellobiose, D-xylose, L-arabinose, D-arabinose and D-ribose, and in terms of growth in vitamin-free medium. Strain XZ 92-1^T differed from its closest relative, *C. sequanensis*, in the assimilation reactions with L-sorbose, lactose, melibiose, raffinose, soluble starch, D-ribose, erythritol, methyl α -D-glucoside, hexadecane and L-lysine and in terms of growth in vitamin-free medium.

The molecular and physiological comparison demonstrated that strains XZ 41-6^T and XZ 92-1^T represent two distinct

novel ascomycetous yeast species. As sexual states were not observed in cultures of single strains or in mating tests (between strains XZ 41-6^T and XZ 69C3) on various media, including potato dextrose agar, 5 % malt extract agar, yeast carbon base (YCB) agar, cornmeal agar, acetate agar and dilute V8 agar (Yarrow, 1998), they were assigned to the genus *Candida* Berkhout according to the current taxonomy of yeasts (Kurtzman, 1998; Meyer *et al.*, 1998). Therefore, we propose the names *Candida tibetensis* sp. nov. and *Candida linzhiensis* sp. nov. for strains XZ 41-6^T and XZ 92-1^T, respectively.

Latin diagnosis of *Candida tibetensis* Bai & Wu sp. nov.

In medio liquido YM post dies 3 ad 25 °C, cellulae cylindratae ad bacillaris, 2.5–2.7 × 5.0–21.4 μm, singulae, binae, cate-nulatae vel paniculatae. Per gemmationem multipolarem reproducunt. Post 1 mensem sedimentum et annulus formantur. In agaro YM post 1 mensem ad 25 °C, butyrosa, membrancea, cremea, sordida, glabra, rugosa cum vittatum tuberculum, margo undulata. In agaro farinae Zea mays post dies 7, pseudohyphae fiunt. Ascosporae non fiunt.

Glucosum fermentatur at non galactosum, sucrosum, maltosum, lactosum nec raffinolum. Glucosum, galactosum, sucrosum, maltosum, cellobiosum (lente), trehalosum, melezitolum, D-xylosum, L-arabinosum, D-arabinosum (lente), D-ribosum (lente; infirme), D-glucosaminum, ethanolum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, methyl α -D-glucosidum, salicinum (infirme), acidum succinicum (lente), acidum citricum (infirme), et hexadecanum assimilantur at non L-sorbose, lactosum, melibiosum, raffinolum, inulinum, amyllum solubile, L-rhamnosum, methanolum, erythritolum, galactitolum, acidum DL-lacticum nec inositolum. Ammonium sulfatum, ethylaminum, L-lysinum et cadaverinum assimilantur at non natrum nitrosum nec kalium nitricum. Ad crescentiam vitaminarum externarum non necessariae sunt. Maxima temperatura crescentiae: 28 °C. Materia amyloidea iodophila non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysat. Systema coenzymatis Q-9 adest. Typus depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.3072^T).

Description of *Candida tibetensis* Bai & Wu sp. nov.

Candida tibetensis (ti.be.ten'sis. N.L. fem. adj. *tibetensis* pertaining to Tibet, the geographical origin of the type strain of the species).

In YM broth, after 3 days at 25 °C, the cells are cylindrical to bacilliform, $2.5\text{--}2.7 \times 5.0\text{--}21.4$ μm and occur singly, in pairs, in chains or in branches (Fig. 2a). Budding is multilateral. After 1 month at 25 °C, sediment and a climbing ring are present. On YM agar, after 1 month at 25 °C, streak culture is butyrous, membranous, cream-coloured, raised, dull, smooth and creased with ridged protuberances; the margin is undulating. In Dalmau plate culture on cornmeal agar, pseudohyphae are formed. Sporulation is not observed. Glucose is fermented, but galactose, sucrose,

maltose, lactose and raffinose are not fermented. Glucose, galactose, sucrose, maltose, cellobiose (delayed), trehalose, melezitose, D-xylose, L-arabinose, D-arabinose (delayed), D-ribose (delayed; weak), D-glucosamine, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, methyl α -D-glucoside, salicin (weak), succinic acid (delayed), citric acid (weak) and hexadecane are assimilated; L-sorbose, lactose, melibiose, raffinose, inulin, soluble starch, L-rhamnose, methanol, erythritol, galactitol, DL-lactic acid and inositol are not assimilated. Ammonium sulfate, ethylamine hydrochloride, L-lysine and cadaverine dihydrochloride are assimilated; sodium nitrite and potassium nitrate are not assimilated. Grows in vitamin-free medium. Maximum growth temperature is 28 °C. Starch-like compounds are not produced. Diazonium blue B reaction is negative. Negative for urease activity. The major ubiquinone type is Q-9.

The type strain, XZ 41-6^T (= AS 2.3072^T = CBS 10298^T), was isolated from a flower of an unidentified plant collected in Linzhi District, Tibet, in July 2004.

Latin diagnosis of *Candida linzhiensis* Bai & Wu sp. nov.

In medio liquido YM post dies 3 ad 25 °C, cellulae subglobosae ($2.7\text{--}5.2 \times 3.7\text{--}6.0$ μm), ovoideae ad elongatae ($3.7\text{--}4.5 \times 23.6\text{--}24.8$ μm), singulae, binae vel adhaerentes, blastospora fiunt. Per gemmationem multipolarem reproducentes. Post 1 mensem sedimentum et annulus formantur. In agaro YM post 1 mensem ad 25 °C, butyrosa, viscidulus, cremea, seminitida, glabra cum radiatis et vittatus tuberculum, margo undulata. In agaro farinae Zea mays post dies 7, pseudohyphae et mycelium verum fiunt. Ascospores non fiunt. Glucosum et galactosum (lente; infirme) fermentantur at non sucrosum, maltosum, lactosum nec raffinolum. Glucosum, galactosum, L-sorbose (lente), maltosum, cellobiosum, trehalosum, lactosum (lente), melibiosum (lente; infirme), raffinolum (lente; infirme), D-xylosum, L-arabinosum, D-ribosum (lente),

Fig. 2. Vegetative cells of *C. tibetensis* XZ 41-6^T (a) and *C. linzhiensis* XZ 92-1^T (b) grown in YM broth for 3 days at 25 °C. Bars, 10 μm .

ethanolum, glycerolum, erythritolum (lente), ribitolum, D-mannitolum, D-glucitolum, methyl α -D-glucosidum, salicinum (lente) et acidum succinicum (lente) assimilantur at non sucrosum, melezitoseum, inulinum, amyllum solubile, D-arabinosum, L-rhamnosum, D-glucosaminum, methanolum, galactitolum, acidum DL-lacticum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum, ethylaminum, et cadaverinum assimilantur at non natrum nitrosum, kalium nitricum nec L-lysinum. Ad crescentiam vitaminarum externarum non necessariae sunt. Maxima temperatura crescentiae: 28 °C. Materia amyloidea iodophila non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysat. Systema coenzymatis Q-8 adest. Typus depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.3073^T).

Description of *Candida linzhiensis* Bai & Wu sp. nov.

Candida linzhiensis (lin.zhi.en'sis. N.L. fem. adj. *linzhiensis* pertaining to Linzhi, the geographical origin of the type strain of the species).

In YM broth, after 3 days at 25 °C, the cells are subglobose (2.7–5.2 × 3.7–6.0 µm) to ovoidal to elongate (3.7–4.5 × 23.6–24.8 µm) and occur singly, in pairs or in groups (Fig. 2b). Elongated cells are straight or curved and may produce small denticles that bear blastoconidia. Budding is multilateral. After 1 month at 25 °C, sediment and a climbing ring are present. On YM agar after 1 month at 25 °C, streak culture is butyrous, viscous, cream-coloured, low and raised, semi-glossy and smooth with faint striations as well as ridged protuberances; the margin is slightly undulating. In Dalmau plate culture on cornmeal agar, pseudohyphae and true hyphae are formed. Sporulation not observed. Glucose and galactose (delayed; weak) are fermented; sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, L-sorbose (delayed), maltose, cellobiose, trehalose, lactose (delayed), melibiose (delayed; weak), raffinose (delayed; weak), D-xylose, L-arabinose, D-ribose (delayed), ethanol, glycerol, erythritol (delayed), ribitol, D-mannitol, D-glucitol, methyl α -D-glucoside, salicin (delayed) and succinic acid (delayed) are assimilated; sucrose, melezitose, inulin, soluble starch, D-arabino-
se, L-rhamnose, D-glucosamine, methanol, galactitol, DL-lactic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, ethylamine hydrochloride and cadaverine dihydrochloride are assimilated; sodium nitrite, potassium nitrate and L-lysine are not assimilated. Grows in vitamin-free medium, but growth is weak and delayed. Maximum growth temperature is 28 °C. Starch-like compounds are not produced. Diazonium blue B reaction is negative. Negative for urease activity. Major ubiquinone type is Q-8.

The type strain, XZ 92-1^T (=AS 2.3073^T=CBS 10299^T), was isolated from a fruit of an unidentified plant collected in Linzhi District, Tibet, in July 2004.

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